MULTIVALENT DENGUE VIRUS VACCINE

This application is a divisional application of U.S. application Ser. No. 09/535,117 filed on Mar. 24, 2000, now U.S. Pat. No. 6,638,514, issued Oct. 28, 2003, and further claims the benefit of priority under 35 U.S.C. § 119(e) from U.S. application Ser. No. 60/126,313 filed on Mar. 26, 1999, and U.S. application Ser. No. 60/181,724 filed on Feb. 11, 2000.

INTRODUCTION

Dengue fever is caused by any of four serotypes of dengue virus, dengue-1, dengue-2, dengue-3, and dengue-4, which are transmitted to humans by mosquitoes. In adults, dengue infections typically cause self-limited but incapacitating acute illness with fever, muscle pains, headache and an occasional rash. The illness may be complicated by hemorrhagic fever, which may be manifested by a positive tourniquet test, spontaneous petechiae, frank bleeding, and/or shock. Dengue hemorrhagic fever is fatal in about 0.5% of cases. Patients who have antibody from an earlier dengue infection who are subsequently infected by another dengue strain have been shown to be at higher risk for dengue hemorrhagic fever.

The mosquito vectors of dengue viruses are found in all tropical and sub-tropical areas of the world and in some temperate areas of the United States, Europe, Africa, and the Middle East. In recent years, endemic and epidemic dengue infections have occurred in Central and South America, Southeast Asia, India, Africa, the Caribbean and Pacific regions. Vector control is impractical.

An effective vaccine is needed which should confer protection against all four serotypes of dengue.

SUMMARY OF THE INVENTION

The present invention satisfies the need discussed above. The present invention relates to vaccine compositions comprising attenuated dengue virus from all four serotypes. The attenuated virus is provided in an amount sufficient to induce an immune response in a human host, in conjunction with a physiologically acceptable vehicle and may optionally include an adjuvant to enhance the immune response of the host.

Therefore, it is one object of the present invention to provide an attenuated dengue virus composition comprising attenuated more than one dengue virus selected from the group consisting of dengue-1, dengue-2, dengue-3, and dengue-4, in any combination.

It is another object of the present invention to provide methods for stimulating the immune system of an individual to induce protection against dengue virus. These methods comprise administering to the individual an immunologically sufficient amount of dengue virus from all four serotypes which have been attenuated by serial passage.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**: Occurrence of >Grade 1 symptoms as a result of vaccine administration.

FIG. **2**: Frequency of distribution of reactogenicity index by serotype.

FIG. **3**: Table showing results of dose-ranging tetravalent dengue vaccine studies.

FIG. **4**: Table showing Immunogenicity of full-dose tetravalent dengue vaccine in 10 subjects.

FIG. **5**: Table showing details of selected formulations of tetravalent vaccine studies.

FIG. **6**, A–H: Interferon γ production by PBMC collected from vaccine volunteers and stimulated with serotype specific virus. All volunteers received only one serotype of vaccine. Graphs on the left (A–D) show results from volunteers that were given the second dose around day 32. Graphs on the right (E–H) show results from volunteers that received the second dose around day 92. A response over 1000 pg/ml was seen just prior to the second dose in most volunteers. Only four volunteers had a response over 1000 pg/ml within the first 15 days of receiving the first vaccine dose.

FIG. **7**, A–D: Interferon γ production of PBMC collected from vaccine volunteers receiving tetravalent vaccine. The PBMC were stimulated individually with each serotype of virus. Individual lines in each graph represent responses of one volunteer's PBMC to individual serotypes of virus. As with the monovalent vaccine recipients, late responses were noted.

FIG. **8**, A and B: Granzyme B mRNA production of PBMC collected from monovalent and tetravalent vaccine volunteers. Cells were collected from all individuals whose PBMC secreted ≧1000 pg IFNγ/ml at any time. This is a semiquantitative representation of the amount of mRNA detected by RTPCR. The upper chart (A) describes the intensity of bands seen for all samples. The lower gel (B) is from selected volunteers to show examples of positive and negative RTPCR assays.

DETAILED DESCRIPTION

The present invention provides attenuated dengue virus of all four serotypes suitable for vaccine use in humans. The dengue viruses described herein were produced by serial passaging of an infectious dengue virus isolate in a suitable host cell line such as primary dog kidney cells so that mutations accumulate that confer attenuation on the isolate. Serial passaging refers to the infection of a cell line with a virus isolate, the recovery of the viral progeny from the host cells, and the subsequent infection of host cells with the viral progeny to generate the next passage.

Preferably, the following attenuated viruses are used in the compositions of the present invention even though other virus compositions, of any of the serotypes, whether attenuated or inactivated, can be used in combination with the attenuated strains described in the present invention. The attenuated dengue-1 virus, derived from 45AZ5 isolate, PDK 20, was deposited on Apr. 30, 1999 under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC) of 10801 University Boulevard, Manassas, Va. 20110-2209, U.S.A., and granted the accession number of VR-2648. The attenuated dengue-1 virus, derived from 45AZ5 isolate, PDK 27, was deposited on Nov. 21, 2002 under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC) of 10801 University Boulevard, Manassas, Va. 20110-2209, U.S.A., and granted the accession number of PTA-4810.

The attenuated dengue-2 virus derived from S16803 isolate, was deposited on Apr. 30, 1999 under the terms of the Budapest Treaty with the American Type Culture Collection (ATCC) of 10801 University Boulevard, Manassas, Va. 20110-2209, U.S.A., and granted the accession number of VR-2653.

The attenuated dengue-3 virus derived from CH53489 isolate, was deposited on Apr. 30, 1999 under the terms of the Budapest Treaty with the American Type Culture Col-